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FURTHER RESEARCH ON IRRADIATED BACON

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INTRODUCTION

THIS paper presents additional data on production of bacon of high quality and consumer acceptance, free from confirmable concentrations of nitrosamines (NA), processed without nitrite or greatly reduced nitrite addition, while providing protection against C. botulinum by irradiation. Previous results of this series of investigations have been reported, including presentations at these Meat Research Worker Meetings (1-6). The objectives of this investigation were to determine: (a) level of irradiation necessary for the microbiological safety of raw, vacuum-packed, smoked bacon pumped with 0 and 40 ppm added nitrite stored under refrigeration and at an abused temperature of 27°C; (b) radiolysis products, and free radical formation and possible reactions in irradiated bacon in order to obtain information required for the regulatory approval of irradiation processed bacon for human consumption based on the chemiclearance principle (7); (c) shelf stability of irradiated bacon stored under incandescent light at 5° ± 1°C, including fat oxidation changes, if any; and (d) sensory quality to assess the reproducibility of the process in comparison with the previous five experiments on irradiated bacon. The microbiological and chemical studies are still in progress and will be reported separately elsewhere by the principal investigators from the Natick Laboratories conducting this work under a research agreement with the Eastern Regional Research Center (ERRC). However, some of the results obtained to date will be reported briefly in this paper, in order to cover the overall scope of the investigation.

EXPERIMENTAL

PREPARATION of bacon and methods of chemical and sensory evaluation were the same as reported previously (4,5). Three lots of bacon were processed in a commercial plant and delivered within 3 hours after slicing to the Natick Laboratory for microbiological inoculation with C. botulinum, vacuum-packaging, and irradiation the day after packaging, using the Natick electron accelerator, with the doses of 0, 5, 10, and 15 kGy at 5°C (1 kGy = 100 Krad). The compositions of the three cures, with calculations based on a 12.5% pump and on 11% pickle retention are shown in Table 1. Pumped belly yields of 112.5%, and the intended smoked yields of 100% were obtained within the normal variations for individual bacon bellies (Table 2). However, the pickle retention, as given by the drained yields, was less than the intended 111%.

In our previous experiments, it was established that under pilot scale production added nitrite can be reduced from 120 to 20 ppm and the desirable color and flavor in the product are still maintained (4,5). The present

experiment was carried out with 40 ppm nitrite to assure sufficient nitrite in the product for good color development (8), even if pumped with equipment not providing good control over ingoing cure solution. Sucrose concentration was reduced to 0.25% ("low sugar") from the 0.75% ("high sugar") used in previous studies (1-6) to obtain information on the microbiological safety of irradiated bacon cured with "low sugar." At the time of slicing (18 slices per pound), three slices were removed from three sections of each belly (brisket, center, flank) in each lot and blended together for proximate analyses. The results are shown in Table 3. Salt content (which is the most reliable index for cure distribution within bacon bellies) is close to the intended addition of 1.5%. It should be emphasized that the product represents a "mild" cured bacon since the salt content is low compared to the concentrations found in commercial products. With this salt content, the water activity of the product is higher than 0.93, which is needed to inhibit the growth of *C. botulinum* (9).

RESULTS AND DISCUSSION

Fat oxidation indices. In a previous study (5) irradiation of bacon containing 20 ppm nitrite at dose levels of 2.2 to 15 kGy at 5°C did not cause changes in the fat oxidation indices (TBA, PV, FFA) due to irradiation and post-irradiation storage in a refrigerator, in the dark up to 25 days, the longest time investigated. In the present experiment, no-nitrite and 40 ppm nitrite bacon, non-irradiated and 5 and 15 kGy irradiated, along with the non-irradiated reference bacon sample cured with 120 ppm nitrite, were stored up to 60 days at 5° ± 1°C in a display case under continuous incandescent illumination. Both the irradiated and non-irradiated vacuum-packaged samples, showed very low TBA and PV values (Table 4). Irradiation slightly increased the PV values which, however, dropped to zero with storage time. There was only a minor increase in FFA in irradiated samples. However, in non-irradiated samples, regardless of nitrite content, the FFA increased about three-fold during storage for 60 days, indicative that the lipases originated in the increasing microbial population rather than being inherent in the bacon.

In the course of previous experiments with irradiated bacon, it was observed that samples that lost vacuum because of damage to integrity of the packaging ("leakers") showed green-gray discoloration and developed a rancid odor. Therefore, an additional experiment was performed. The commercial 1-lb. bacon samples were used in which holes were punched with a 3 mm metal probe in the center of the packages before and after irradiation with 15 kGy at 5°C. After storage in the display case under light for 5 days the punched packages showed distinct gray discoloration surrounding the holes and the spots emitted a distinct rancid odor that was noticed by experienced food technologists (Table 5). In the samples punched before irradiation, the gray discoloration was observed immediately following irradiation treatment. TBA and PV values increased. The increase appears to be small because the entire 1-lb. samples were homogenized instead of using the discolored rings only, for the TBA and PV determinations. This simple experiment indicates that in commercial production of irradiated bacon, the "leakers" can be detected immediately after irradiation by formation of gray discoloration, removed prior to sale, and used as "rework" in other processed meat products, as is a normal commercial practice.
Sensory quality. In previous experiments with irradiated bacon it was shown that irradiation of bacon with 5, 7.5, 15, and 30 kGy at 5°C and with 30 kGy at -30°C resulted in products of high consumer acceptance, with high

scores for odor, flavor, and texture (1-5). In most instances the bacon cured without nitrite and with 20 or 40 ppm nitrite scored at the same level for odor, flavor, and texture as the bacon cured with the 120 ppm commercial level of nitrite, indicating that in case of bacon, nitrite is not needed for odor and flavor development. The only differences observed were in color when compared with the pink pigment of the nitrite-cured bacon (1-5).

In the present study the effect of irradiation on color and odor was studied when the product was stored in a display case under light up to 60 days (Table 6). The color and odor scores of five and above indicate acceptable products. The color scores during the first test (20 days storage) indicated that 40 ppm nitrite was sufficient to equal the color intensity of the 120 ppm nitrite cured bacon. They further indicate that irradiation increased the color intensity of the bacon cured without nitrite, although the results are less pronounced than observed in the previous experiments (4,5). It is now well established that irradiation restores the original red color of the raw bacon by reducing metmyoglobin, the brown color of processed bacon cured without nitrite, to deoxymyoglobin (10), the pigment of the raw uncured fresh bacon.

There were no significant differences in odor among all samples stored for 20 days under the light, and the products did not show any detectable off-odor. However, after 30 days storage, the odor scores for the non-irradiated nitrite-free bacon dropped below the acceptable level and was described by panelists as putrid; after 60 days storage, the odor score was slightly higher but still objectionable and the off-odor detected was described as being putrid-sour. The non-irradiated nitrite-cured bacon, both with 40 and 120 ppm nitrite, showed decreased odor scores after 30 days storage; the odor scores dropped below the acceptable level after 60 days storage. The nature of the off-odor, as judged by the panelists, was definitely sour after this storage period. The irradiated samples, both without nitrite and cured with 40 ppm nitrite, maintained acceptable odor scores throughout the 60 days storage, and were described as having normal, smoked bacon odor.

Consumer acceptance. Consumer acceptance was evaluated with volunteer taste testers at ERRC using the 9-point hedonic scale on the product stored for 22 days. The bacon containing 40 ppm nitrite, both irradiated and non-irradiated, scored as high as the 120 ppm nitrite not-irradiated bacon at the P <.05 and <.01 significance levels, respectively (Table 7). Only nitrite-free, non-irradiated bacon was scored significantly lower than the non-irradiated 120 ppm nitrite bacon at the P <.05 level. No other acceptance tests were conducted on the bacon samples in this study, since sufficient data on the sensory quality of nitrite-free and low-nitrite bacon have been obtained previously (3-5). This evaluation was performed only to confirm that high quality irradiated bacon cured without nitrite, or with low nitrite, can be consistantly produced by a commercial processor.

Nitrosamines. Nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR), the principal volatile nitrosamines, were present in fried non-irradiated bacon in each of the five previous experiments (1-4). In general, nitrite-free bacon contained non-detectable (N.D.) to 1 ppb NDMA and N.D. to 3 ppb NPYR in the fried edible portion. In the bacon samples cured with 20 or 40 ppm nitrite, the NDMA was in the same range (N.D. to 1 ppb) and NPYR from N.D. to 5 ppb. Since nitrite-free bacon occasionally had detectable NPYR, possibly due to nitrite produced from nitrate naturally found in raw pork (5 to 15 ppm), or resulting from contamination from the tap water and salt used for the cure preparation, or during the smoking process, a special study was conducted

using distilled water to dissolve the curing ingredients and using the matched bellies, subdivided into three sections, flank, center, and brisket. The results obtained (6,11) showed the following: a) there were no nitrosamines in nitrite-free or 20 ppm nitrite bacon; b) there was no residual nitrite in 20 ppm or in 120 ppm nitrite cured irradiated bacon; c) in 120 ppm nitrite cured bacon the level of NDMA averaged only 0.89 ppb vs 0 ppb for nitrite-free bacon, the amount of NPYR was only about one third (3.39 ppb) of the amount found in non-irradiated reference bacon samples (9.28 ppb); d) irradiation (30 kGy at -40°C) destroyed from 79 to 92% added NDMA and 69 to 88% added NPYR, thus suggesting that irradiation has a destructive effect on preformed nitrosamines (11).

In all previous studies on nitrosamine determination in bacon, the irradiation was performed on frozen samples, since the general consensus is that irradiation in the frozen state produces less radiolysis products (12), thus improving the product flavor and acceptance. This is generally true for most irradiation sterilized meats (13), but in the case of bacon, irradiation can be applied to the chilled product at 5°C, without affecting the product quality (unpublished data for 30 kGy irradiated bacon at 5° vs -30°C).

The effect of irradiation temperature on nitrosamine formation was investigated on five bacon samples cured with 40 ppm nitrite and irradiated at +5° or -40°C. Each bacon sample consisted of slices taken from the brisket, flank, and center portions of three different bacon bellies; subdivided into three sections, one slice from each location being taken for the +5°C irradiation (30 kGy) and the adjacent slices for the -40°C irradiation. The composite samples consisted of 9 slices of about 0.5 total weight. The NDMA and NPYR analyses were performed on the fried portions and the fat drippings (11). The results are shown in Table 8. The paired comparison (t-test) showed no statistical difference between nitrosamines formed in bacon irradiated at two temperatures. Since the nitrosamine levels in 40 ppm nitrite bacon are very low (Table 8) additional experiments are required to verify the results in order to determine the precise effect of the irradiation process at the lower doses (10 to 20 kGy), anticipated for use with vacuum-packed fresh bacon irradiated at refrigerated temperatures.

Microbiological safety. Previous studies showed that irradiation with 2.2 kGy was insufficient to destroy common spoilage microorganisms in bacon, while 7.5 kGy was sufficient for this purpose (4,5). To investigate further the effect of nitrite concentration and irradiation on the microbial flora of bacon, aerobic mesophiles, yeasts, enterococci, staphylococci, lactobacilli, and coliforms were determined in non-irradiated 0, 40, and 120 ppm nitrite bacon and in 0 and 40 ppm nitrite bacon irradiated with 5, 10, and 15 kGy at +5°C. The microbiological studies were conducted on the vacuum-packed bacon samples stored for up to 60 days at 5°C and at 27°C. The preliminary data indicate that in 5 kGy irradiated samples some microorganisms survived, while 10 kGy resulted in sterile products free from all bacon spoilage microorganisms for up to 61 days storage (the end of the experiment) (14). In bacon samples with inoculated C. botulinum spores types A and B, irradiation with 15 kGy provided protection against botulinum toxin production for up to 61 days in the nitrite-free cured bacon; the 40 ppm nitrite bacon had a few toxic samples after 61 days incubation. All the inoculated, non-irradiated bacon containing 120 ppm nitrite (reference sample) stored at 27°C developed swollen and toxic samples. It is interesting to note, that in the previous inoculated bacon pack study, when 0.75% sucrose was

used, the 120 ppm nitrite cured, non-irradiated bacon had only two swollen and one toxic sample after 60 days incubation (16). Also the use of the "high sugar" (0.75%) apparently inhibited C. botulinum spore outgrowth in the bacon cured with the low nitrite level and irradiated with a dose lower than 15 kGy. In the present experiment, when 40 ppm nitrite and 0.25% sucrose were used, most of the samples irradiated with 10 kGy produced swells and toxin after 17 days, whereas in the previous experiment, when 20 ppm nitrite and 0.75% sucrose were used, the 10 kGy irradiated samples contained only nine swollen and five toxic of the total 20 inoculated samples after 61 days incubation at 27°C. Since sucrose utilizing lactobacilli are destroyed by irradiation at 10 kGy, the pH level inhibitory for the C. botulinum growth and toxin formation may not be reached. Sucrose also might have an effect on the repair of irradiation injured spores of C. botulinum similar to the effect observed for NaCl (15). Additional study must be done to determine importance of sugar for microbiological safety of irradiated bacon. A more detailed report on the microbiological safety of irradiated bacon will be published in a separate paper. However, at this time it appears that for vacuum-packed fresh bacon stored and distributed under refrigeration, an irradiation dose of 15 kGy is needed to provide protection against C. botulinum under incidence of abuse temperatures during marketing and distribution (16).

Chemical study. The effects of Co^{60} and electron irradiation with doses 5, 15, and 30 kGy at 5°C and -40°C on the volatile radiolysis products and non-volatile compounds resulting from irradiation of bacon cured with 40 ppm nitrite were investigated. ESR spectroscopy was also used to determine free radical formation. These studies have not been completed but the data accumulated indicate that the irradiation products are the same as those found in other meats (beef, pork, chicken, ham) (7,12,17). The preliminary data on the ESR investigation on irradiated bacon showed a typical ESR spectrum for triglycerides in bacon irradiated with 30 kGy at low temperature (-40° to -160°C). These however, disappeared during defrosting of the samples (18). No ESR spectra (i.e., no free radicals) are expected in bacon irradiated with doses of 15 and 30 kGy at 5°C; this, however, is being investigated further. We wish to determine whether the radiolysis products and the free radical formation and their decay in irradiated bacon are qualitatively the same as those found for irradiated chicken, pork, and beef and, consequently, whether the effect of irradiation within specified irradiation parameters (dose, tem perature), is the same for irradiated bacon as for other meats. The chemical data from other irradiated meats are available (7,12,17), and for one meat (chicken) a thorough toxicological study including the animal feeding experiments and related studies (mutagenicity, teretogenicity, protein efficiency, etc.) is nearly complete. Since we will soon have the results of acute toxicity studies on irradiation sterilized chicken, conducted on mice and dogs over a 3-year period (19), we do not anticipate needing similar studies on bacon. We anticipated that by using the chemical data and applying the chemiclearance principle (7), irradiated bacon will be accepted for human consumption by health authorities.

PRELIMINARY CONCLUSIONS

1. Irradiation effectively destroys C. botulinum, thus allowing production of "mild cured bacon" either without nitrite or with greatly reduced nitrite. 10 kGy irradiation appears to be sufficient for destruction of indigenous microflora in bacon, whereas 15 kGy appears to provide sufficient protection against C. botulinum during abuse temperature storage (27°C). The protective effect of irradiation with 10 to 15 kGy against C. botulinum in 0 and 20-40 ppm nitrite bacon is greater than 120 ppm nitrite in non-irradiated bacon.

2. Based on the results of all studies on bacon, it is concluded that an addition of nitrite is not needed for acceptable odor and flavor of the product. For the production of the characteristic color of the product after frying, 20 to 40 ppm nitrite addition is sufficient.

3. Treatment of bacon with 10 or 15 kGy of Co^{60} gamma ray irradiation, or electron irradiation, can be applied to conventionally vacuum-packed products under conventional refrigeration. Freezing of the product for irradiation is not required to obtain irradiated products of good quality.

4. The irradiated product contains practically no residual nitrite and undetectable or only traces of nitrosamines. Irradiation shows promise as being one of the most efficient alternatives for elimination or reduction of ingoing nitrite in processed bacon.

5. Animal feeding studies on irradiated bacon to demonstrate its wholesomeness may not be necessary. Clearance of the irradiated bacon by the health authorities for human consumption is anticipated based on the microbiological and chemical data, and applying the chemiclearance principle.

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Table 1. Intended additions of curing components to raw smoked bacon*

Lot	NaCl %	Sucrose %	Na-TPP %	Na-Eryth. ppm	$NaNO_2$ ppm
A	1.5	0.25	0.3	550	0
B	1.5	0.25	0.3	550	40
C	1.5	0.25	0.3	550	120

*Calculated on 12.5% pump and 11% pickle retention.

Table 2. Processing yield data

Processing steps: Number bellies/lot	A 17	B 23	C 8
Raw weight, range (lbs.)	10.4-12.6	10.2-13.3	10.0-12.2
Raw weight, ave. (lbs.)	11.3	11.5	11.3
Pumped weight, range (%)	110.1-116.1	110.8-114.8	108.8-113.9
Pumped weight, ave. (%)	112.0	112.6	112.5
Drained weight, range (%)	104.7-111.6	105.4-110.9	108.5-112.1
Drained weight, ave. (%)	107.5	108.7	110.1
Smoked weight, range (%)	93.3-103.4	96.2-103.8	18.1-102.9
Smoked weight, ave. (%)	99.6	100.3	100.6

Table 3. Proximate composition of the product

Lot	Added $NaNO_2$ ppm	Proximate Composition H_2O %	Protein %	Fat %	NaCl %	P %	pH	A_w
A	0	38.5	10.7	47.7	1.80	.17	6.00	.94
B	40	36.7	10.7	50.0	1.55	.16	6.00	.95
C	120	32.8	8.5	55.8	1.49	.15	6.10	.95

Table 4. Effect of irradiation and storage on fat oxidation indeces of raw smoked bacon, vacuum-packed, and stored under light of 5°C ± 1°C

Lot	Fat Oxidn. Index	0 kGy			5 kGy			15 kGy		
		1	30	60	1	30	60	1	30	60*
A (0 $NaNO_2$)	TAB[1]	.53	.44	.39	.48	.54	.62	.43	.52	.48
	PV[2]	.9	1.0	.7	1.3	.5	0	1.7	.4	0
	FFA[3]	.37	.76	1.1	0.42	.54	0.55	.42	.45	.54
B (40 $NaNO_2$)	TBA	.02	.16	.16	.59	.32	.31	.52	.28	.28
	PV	.6	0	0	.9	0	0	1.2	0	0
	FFA	.32	.68	1.5	.30	.42	.55	.37	.57	.53
C (120 $NaNO_2$)	TBA	.70	.14	.12	-	-	-	-	-	-
	PV	.9	0	0	-	-	-	-	-	-
	FFA	.40	.74	1.2	-	-	-	-	-	-

[1] TBA = mg malonaldehyde per 1,000 g sample
[2] PV = peroxide value, meqs. O_2 per Kg fat
[3] FFA = free fatty acids as % oleic acid in the fat
*Days of storage under light of 5° ± 1°C

Table 5. Effect of loss of vacuum on TBA, PV, and color and odor of irradiated and non-irradiated bacon stored under light for 5 days at 5°C ± 1°C

Packaging damage	Samples per treatment	TBA	PV	Color	Odor
None	1	.35	0.0	normal	normal
(Good vacuum)	2	.27	0.2	pink	fresh bacon
3 mm hole	1	1.03	0.9	5 cm gray, 10 cm gray	slightly
in the center	2	1.52	2.6	ring discoloration	rancid
before irrad.[1]					
3 mm hole	1	.22	1.0	5 cm gray ring	slightly rancid
in the center	2	.45	1.3	discoloration	at discolored spots
after irrad.[1]					

[1] Irradiated with 15 kGy at 5°C (1 lb. commercial bacon samples, assumed cured with 120 ppm $NaNO_2$).

Table 6. Effect of irradiation on color and odor of raw smoked bacon, vacuum-packed and stored under light at 5°C ± 1°C

Lot	kGy at 5°C	Color 20	Color 30	Color 60	Odor 20	Odor 30	Odor 60[1]
A	0	5.3[c]	5.1[c]	6.7[a]	7.0	2.7[d]	3.2[c]
(0 $NaNO_2$)	5	5.4[c]	6.9[ab]	6.2[abc]	6.2	7.1[a]	5.8[a]
	15	6.2[bc]	*	6.0[abc]	6.3	*	5.8[a]
B	0	7.6[a]	7.7[a]	6.8[a]	7.6	4.4[c]	3.8[bc]
(40 $NaNO_2$)	5	7.0[ab]	6.1[bc]	5.5[bcd]	7.5	7.3[a]	5.9[a]
	15	7.4[a]	7.2[a]	5.4[bc]	7.5	7.5[a]	5.8[a]
C	0	7.3[ab]	6.2[bc]	4.9[d]	7.6	5.3[bc]	4.2[bc]
(120 $NaNO_2$)							
LSD (<.05)		1.1	1.1	1.0	NSD	1.5	1.0

[1] Days of storage under light at 5°C ± 1°C.
*Discarded sample ("leaker" = lost vacuum).
abcd - Means within the columns with the same letters are not statistically different.
NSD = no significant difference.

Table 7. Effect of nitrite level and irradiation on preference of bacon after frying

Acceptance Test, 9-point hedonic scale; n = 25
(ERRC Preference Taste Panel)

Lot	Added $NaNO_2$ ppm	kGy at 5°C	Mean	P(<.05)	(P<.01)
A	0	0	5.2	B	A
	0	15	5.7	AB	A
B	40	0	6.2	AB	A
	40	15	6.2	AB	A
C	120	0	6.5	A	A

Means with the same letter are not statistically different. (Vacuum-packed bacon sample stored in refrigerator for 22 days at 4° ± 1°C).

Table 8. Effect of 30 kGy Co^{60} gamma irradiation on the nitrosamine content in bacon cured with 40 ppm added nitrite and irradiated at +5 vs -40°C

	Fried Portions (ppb)				Drippings (ppb)			
	+5°C		-40°C		+5°C		-40°C	
Sample no.	NDMA	NPYR	NDMA	NPYR	NDMA	NPYR	NDMA	NPYR
1	1	3	N.D.	4	1	4	1	5
2	1	5	N.D.	4	1	8	2	7
3	N.D.	2	N.D.	2	2	5	3	10
4	N.D.	2	N.D.	1	1	4	1	5
5	1	2	1	1	1	4	1	5
Ave.	0.6	2.8	0.2	2.4	1.2	5.0	1.6	6.4

Paired t-test: no significant difference between the +5° and -40° irradiation temperatures (df = 4).